REMARKS

With the entry of the present Amendment, claims 1-26 and 28-30 are in this application. Claims 1-29 have been examined. Claims 24-29 have been renumbered as 25-30 (the as-filed claims included two claims numbered "24"). Claims 1, 2, 8, 9, 14, 16, 18-20, 24, 25, 27 and 28 have been amended to correct inadvertent typographical and clerical errors. Claims 1 and 26 have been amended to recite that the ARPs are "heparin binding"; this language is supported by the paragraph that bridges pages 28 and 29 of the as-filed Specification. Claim 27 (as-filed 26) has been canceled without prejudice. The Specification has been amended at page 28-29 to complete a citation. None of the amendments made herein constitutes the addition of new matter.

The Information Disclosure Statement

Applicants respectfully note that an electronic Information Disclosure Statement was filed by the undersigned on October 7, 2004, and that this submission was acknowledged on that date by the United States Patent and Trademark Office. Paper copies of the Acknowledgement and the Information Disclosure Statement are provided herewith.

Applicants respectfully request that the Examiner consider the United States patent references and that he initial and return a copy of the list of United States patent references cited on the electronic Information Disclosure Statement.

The Objections to Certain Claims

Claim 2 was objected to for containing extraneous text. This claim has been amended to remove the extraneous text.

Claims 8, 9 and 26 were objected to as being in improper form. Claims 8, 9 and 26 were amended to properly recite dependencies. It is believed that these claims as amended further limit the claims from which they depend.

Claims 24 and 27 are alleged to be substantial duplicates. As-filed claim 27 (now 28) has been amended to depend from claim 26 rather than from claim 17. It is believed that these claims are no longer substantial duplicates in that claim 28 encompasses a contacting step not embodied in claim 17.

Claim 14 has been amended to correctly state three parameters.

Claim 16 has been amended to depend from claim 14, as suggested by the Examiner.

Claim 18 has been amended to depend from claim 17, rather than from claim 11, as correctly noted by the Examiner.

Claim 29 (now claim 30) has been amended to remove the spurious recitation "any of".

In view of the amendments to the claims to properly recite dependencies and to correct inadvertent clerical errors, approval of the claims is now requested.

The Rejection under 35 U.S.C. 102(b)

Claims 17, 24 and 27 have been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by, or in the alternative under 35 US.C. 103(a) as allegedly obvious over Pushko et al. (1997). Applicants respectfully traverse this rejection.

The Pushko reference is said to teach methods of preparing alphavirus replicon particles (ARPs) comprising introducing an alphavirus replicon vector and one or more helper nucleic acid molecules into alphavirus-permissive cells via electroporation. The Patent Office has conceded that the cited reference does not teach a specific concentration range of permissive cells during electroporation or the concentration of the replicon vector. The Examiner has alleged that "one would know that such concentrations can be optimized".

As noted above, the Patent Office has acknowledged that the Pushko reference does not teach the concentrations of cells and nucleic acid. Thus, this reference does not teach every limitation of claims 17, 24 and 28 (formerly 27). Accordingly, the rejection under 35 U.S.C. 102(b) is not proper and must be withdrawn.

Applicants respectfully point out the cited Pushko reference does not teach a salt wash step ("contacting the modified host cells after step (b) with an aqueous solution having an ionic strength of from 0.2 M to 5 M to release the ARPs into the aqueous solution to produce a ARP-containing solution"), as recited in step (c) of claim 25, from which claim 28 depends. Accordingly, the Pushko reference does not teach every limitation of claim 28 (formerly 27), and therefore it cannot be properly deemed to anticipate claim 28 of the present application.

With respect to alleged obviousness of claims 17, 24 and 27 over the cited Pushko reference, Applicants respectfully maintain that there is no teaching or suggestion that particle yield could be or would be improved with the utilization of the particular host cell and nucleic acid concentrations specified in claim 17.

In the as-filed Specification in the paragraph bridging pages 23 and 24, the typical cell density range in electroporation mixtures is discussed.

> Additionally, the density of the cells is also a factor, and manufacturers' recommendations are between 1-5 x 10^6 cells/mL for Vero and NIH-3T3 cells, and slightly higher for BHK and CHO cells, both of which are smaller than Vero cells (see, e.g. Multiporator® Cuvette Manual, Brinkmann, Westbury, NY; Genetronics, San Diego, CA (BTX Division) protocols for the ElectroCell Manipulator (ECM®) or the ElectroSquarePorator™; Parham, J. et al. 1999 *CytoTechnology* 28:1-9). The art teaches that higher cell densities than those recommended result in non-homogenous field conditions in the electroporation milleu, which can lead to cell fusion. Liljestrom and Garoff, *J. Virology* 65:4107-4113, 1991, used electroporation to introduce a single, capped RNA helper and a Semliki Forest Virus replicon RNA into BHK cells at a concentration of 5 x 10^6 cells/mL.

Thus, the art teaches the use of significantly lower cell densities than those specified in claims 17 and 26 (as renumbered), from which claim 28 (as renumbered) now depends. Accordingly, Applicants respectfully submit that the art in fact teaches away from the present invention as claimed.

Neither does the cited reference teach or suggest that contacting the cells in which the viral particles were produced with an aqueous solution of from 0.2 to 5 M ionic strength as specified in step (c) of claim 1 and step (c) of claim 25, from which claim 27 now depends, could or would improve ARP yield. Further discussion of the references is provided below.

In view of the foregoing, Applicants respectfully maintain that the claims 17, 24 and 28 (formerly 27) are not made obvious by the teachings of the cited Pushko reference, and withdrawal of the rejection is requested.

The Rejections under 35 U.S.C. 103(a)

Claims 1, 3-12, 14-27 and 29 have been rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Pushko in view of Bell et al. (1978). Applicants respectfully traverse this rejection.

The Patent Office has characterized the cited Pushko reference as teaching a replicon vaccine vector system based on an attenuated strain of VEE, where the replicon nucleic acid comprises at least a virus packaging signal and at least one heterologous coding sequence expressible in the alphaviral replicon nucleic acid, where the host cell comprises at least one helper function, to produce a modified host cells. In this case the helper functions were provided either by monopartite or bipartite helper systems. Pushko is said to teach culturing the modified host cell under conditions allowing expression of the at least one helper function, allowing replication of the alphavirus replicon nucleic acid and packaging of the alphaviral nucleic acid to form ARPs. The Patent Office has acknowledged that the cited Pushko reference does not teach the effect of salt concentration on the modified host cell after the culturing step to release the ARPS to produce an ARP-containing solution.

The Bell reference is said to teach the effect of salt concentration on the release of alphavirus particles in cell culture systems. Bell described results from cell culture systems used to replicate Sindbis virus. Bell reported that "when the NaCl concentration is lowered, maturation of infectious particles and particle release is significantly reduced". The importance of Bell is said to be that "it establishes a dependence between salt concentration and particle release at the point when particles are harvested in cell culture. While Bell does not explicitly teach an optimal salt concentration or range, it is alleged that "one would recognize from Bell that one should optimize the salt concentration because such optimization greatly affects virus particle yield."

The Patent Office further concluded that one of ordinary skill in the art would have been motivated to combine the teachings of Bell and Pushko and that one of ordinary skill in the art would have expected an increased yield of non-infectious particles because the teaching were well-established at the time of applicant's invention. As a point of clarification, Applicants respectfully point out that the alphaviral

particles produced are not "non-infectious"; they can infect a cell, but they are propagation-defective (see page 9, line 31 through page 10, line 14 of the Specification).

Pushko is further said to teach the use of Vero cells as the alphavirus-permissive cells. The Patent Office has added that it would have been obvious to optimize the electroporation mix and the gap between electrodes to ensure transfections of 100% of the cells through routine experimentation. As to claims 10 and 12, the Patent Office has alleged that one would reasonably wash the cells and would perform this wash in a medium of reduced salt to prevent premature release of virus, and one would also remove DNase to remove residual nucleic acid in the media. As to claim ..., per the Patent Office, this again appears to be a matter of routine optimization/experimentation. As to claim 22, which recites purification methods, one would reasonably filter or purify the virus following harvest. The Patent Office has also concluded that the use of capped vs. uncapped RNA transcripts would appear to be within the purview of routine experimentation.

Claims 1 and 26 (as renumbered) have now been amended to recite that the alphavirus replicon nucleic acid is of a heparin-binding alphavirus. This amendment is supported by the as-filed Specification, for example, at the paragraph bridging pages 28-29. Neither of the cited references makes a connection between the ability of a virus or ARP to bind to a heparin (or other glycosaminoglycan) and the improvement in virus or particle yield when the producing cells are subjected to a contacting/release step with a solution having an ionic strength from 0.2 to 5 M.

Applicants respectfully maintain that the cited Bell reference appears to be related to the effect of **growth** medium salt concentration (half-strength v. normal strength) on maturation of virus particles in infected cells, rather than the effect of higher than normal medium salt concentrations in washes **after** virus or particle propagation and maturation. The effects of salt in the present invention is not dependent on

incubation temperature (see page 21, line 32 through page 22, line 2 of the Specification); thus, Applicants conclude that the salt does not stimulate maturation of ARPs, but rather that the contacting step removes (already) mature ARPs from the cells. In addition, it is noted that the Bell reference relates to incubation of the virus particle-producing cells in medium with half the normal concentration of NaCl used in the cell growth medium and then further incubating those cells in medium containing the normal NaCl concentration. Bell only provides a single salt concentration for the normal growth medium, namely 0.138 M, and exactly one-half that concentration, 0.069M, for the low salt medium. Applicants respectfully maintain that the combination of the teachings of the Pushko and Bell references would not have led one of ordinary skill in the art to the present invention as claimed. The courts have cautioned against the impermissible use of hindsight in evaluating patentability, and they have held that it is necessary that the cited references provide the motivation for their combination. See, for example, ACS Hospital Systems, Inc. v. Montfiore Hospital, Inc., 221 U.S.P.Q. 929, C.A.F.C., 1984; Northern Telecom, Inc. v. Datapoint Corp., 15 U.S.P.Q.2d 1321, 1323 (Fed. Cir. 1990); In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992) ("[t]here must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination" and "[t]hat knowledge can not come from the applicant's invention itself."); and In re Dow Chemical Co., 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Neither reference teaches or suggests any washing of the virus- or particle-producing cells in a higher than normal salt solution as a way to improve yield.

In addition, Applicants point to Figure 4, which shows that the salt wash can result in as much as a hundred-fold increase in ARP yield. Applicants respectfully submit that the magnitude of the increase in particle yield is significantly greater than one would expect from mere routine optimization of experimental parameters, and that this magnitude increase points to the inventive nature of Applicant's discovery of the effect of a high-salt wash on the release of heparan-binding alphaviral particles from the cells in which they are produced.

In view of the foregoing, Applicants respectfully maintain that the Patent Office has not established that the claimed invention is *prima facie* case obvious over the cited references, and the rejection must be withdrawn.

Claim 2 has been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Pushko in view of Bell as applied above and further in view of Polo et al. (1999). Applicants respectfully traverse this rejection.

Claim 2 specifies that the at least one helper function is encoded by a nucleic acid sequence incorporated within the genome of the host cells. The cited Pushko and Bell references do not teach this, but the cited Polo reference teaches such a cell. The Patent Office has concluded that one of ordinary skill in the art would have been motivated to apply the teachings of Polo and that the invention would have been obvious to one of ordinary skill in the art at the time the invention was made.

As argued above, neither the Pushko nor the Bell reference teaches or suggests that washing the cells (and/or cell debris) in which the viruses or particles with heparin-binding properties were produced with a solution having an ionic strength from 0.2 to 5 M has a beneficial effect on the virus or particle yield. As argued above, the Bell reference appears to teach a relation between virus maturation and low salt v. normal salt concentration in the culture medium. Again, the present invention entails an ionic strength dependent contacting/release step which is not dependent on temperature or on the presence of growth medium; accordingly, Applicants have concluded that it is a wash (release) rather than a maturation process. The present inventors have made the correlation between the heparin-binding ability and the improved yield with a salt release step. Neither does the cited Polo reference teach or suggest such a step, as required by base claim 1.

Accordingly, Applicants respectfully maintain that claim 2 is not *prima facie* obvious over the cited references, and the rejection must be withdrawn.

Conclusion

Applicants respectfully submit that the pending claims are in condition for allowance and early notification thereof is requested.

If, in the interest of expediting prosecution, the Examiner has questions or comments, he is invited to telephone the undersigned at the indicated telephone number.

This Amendment is accompanied by a Petition for Extension of Time for a two-month period for which the Director is authorized to charge $225.00 to deposit account number 07-6969. If the amount submitted is incorrect, please charge any deficiency or credit any overpayment pursuant to Deposit Account No. 07-1969.

Respectfully submitted,

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